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Beads or microparticles bearing different fluorescent dyes are available commercially for calibration of flow cytometers and fluorescence microscopes. The microparticles, also known as latex particles, are made of polystyrene that are doped with or coupled to a single or a plurality of fluorescence dyes. These calibration beads are used routinely to adjust detector outputs and signal to noise ratios in flow cytometers. The disadvantage with these beads is that they are not magnetic and cannot be used in analytical procedures requiring magnetic beads in analytical platforms. These analytical platforms detect targets that are magnetic and fluorescent. The targets can be made magnetic by labeling targets with magnetic particles conjugated to antibodies specific for targets. The novel beads disclosed in this invention are labeled with both magnetic particles and fluorescent dyes that can be used in the CellSpotter® and CellTracks™ systems as calibration beads.

There are several methods for preparing magnetic calibration beads. One could attach small magnetic particles to commercial calibration beads by absorption or by using conjugation chemistries to couple magnetic particles to fluorescent beads. The sizes of fluorescent beads could range from 1-20um. The magnetic particles for conjugation are preferably less than 0.2um. In the preferred mode, commercially available 6um non-magnetic red beads (Deep red beads from Molecular Probes, Eugene, OR, Part Number L-14819) were made magnetic by direct absorption of protein-coated Immunicon ferrofluids as follows:

Deep red beads ( $5 \times 10^6$  beads/ml) were washed with excess phosphate buffered saline (PBS) by centrifugation to remove any detergent present in the bead sample. Bovine Serum Albumin (BSA) coated magnetic particles (Immunicon, Part Number 6020) were absorbed onto Deep red beads by mixing the washed beads and the magnetic particles at room temperature for 3 hours. The unbound magnetic particles were removed by centrifugation at 300x g where free magnetic particles will stay in the supernatant due to their small size. The magnetic beads were then resuspended in PBS and washed in a magnetic separator (HGMS; Immunicon QMS) to remove beads that were non-magnetic. The beads were then resuspended in PBS. The amino groups on the outer surface of the protein-coated magnetic particles were then crosslinked with excess 0.5% paraformaldehyde (PFA) for 2 hours at room temperature to improve stability of the coated beads. The beads were washed again by centrifugation to remove excess PFA. The beads were then resuspended and stored in PBS with BSA prior to use (PFA also introduces free aldehyde groups which can be utilized for attaching additional protein layers or quenched with a substance containing amino groups, for example, as shown above, with BSA, to minimize bead aggregation). The coating procedure did not alter the physical or fluorescence properties of the beads and thus allowed their use as calibration beads in the CellSpotter and CellTracks instruments.